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Viruses in water

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Attention is drawn in this paper to the increasing problem of viral contamination of water and shellfish, particularly since growing demands for available water resources by a rising world population and expanding industry will make the recycling of wastewater almost inevitable in the future. The problem of eliminating viruses pathogenic for man from water is considered in the light of present water treatment procedures, which are often inadequate for that purpose. Man may be exposed to waterborne viruses through the consumption of contaminated water, shellfish, or crops, as a result of recreational activities involving water, or from aerosols following the spraying of crops with liquid wastes. Physical and chemical methods of eliminating viruses from water are discussed.

It is 30 years since studies on the presence of human enteric viruses in water began in earnest, but the public health significance of these viruses in water has yet to be ascertained. This has been due in part to the inapparent nature of most of the infections caused by these viruses and the lack of methods for their detection in many places. Studies have shown that enteric viruses easily survive present sewage treatment methods and many can persist for several months in natural waters.

In many parts of the world, increased demands on available water resources caused by the concurrent expansion of the population and industrial demand make recycling of domestic wastewater almost inevitable in the future. One of the major problems to be overcome is the development of adequate methods to ensure that viruses pathogenic for man are eliminated from reclaimed water. This problem is also compounded by the concern that present water treatment procedures may not always be sufficient to prevent viruses from reaching community water supplies.

Attention is increasingly being paid to problems of viruses in water and shellfish. However, it seems that the actual scope of this problem has yet to be brought to the

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attention of the medical profession, even though it has far-reaching implications for the spread of viral disease.

VARIETY AND CONCENTRATION OF VIRUSES FOUND IN WATER

More than 100 different enteric viruses are known to be excreted in human faeces. Table 1 lists the major groups of enteric viruses that have been found in raw sewage or are known to be present in the faeces of infected persons, including healthy carriers. More than one million viruses may be excreted per gram of faeces, and concentrations as high as 500 000 infectious virus particles per litre have been detected in raw sewage in some parts of the world. The average enteric virus density in domestic sewage in the United States has been estimated to be about 7000 viruses per litre. The amount of virus present in raw sewage is highly variable, depending on such factors as the level of hygiene in the population, the incidence of disease in the community, the socioeconomic level, and the time of year. In the United States, peak levels occur in the late summer and early autumn. Enteric viruses survive the customary secondary sewage treatment and chlorination, as commonly practised, in sufficient numbers to be isolated easily by modern concentration procedures at all times of the year. It is not surprising, therefore, that these viruses have been detected in several of the major rivers in that country.

Little is known about the occurrence of viruses in drinking water because, until recently, methods for the concentration of viruses from large volumes of water have been lacking. A few reports do exist in the literature. In a study during the 1960s in Paris, enteric viruses

Table 1. Human enteric viruses that may be present in water

Virus group [a]	Number of types	Disease or sign caused
Enteroviruses		
Poliovirus	3	paralysis, meningitis, fever
Echovirus	34	meningitis, respiratory disease, rash, diarrhoea, fever
Coxsackievirus A	24	herpangina, respiratory disease, meningitis, fever
Coxsackievirus B	6	myocarditis, congenital heart anomalies, rash, fever, meningitis, respiratory disease, pleurodynia
New enteroviruses	4	meningitis, encephalitis, respiratory disease, acute haemorrhagic conjunctivitis, fever
Hepatitis type A (probably an enterovirus)	1	infectious hepatitis
Gastroenteritis type A (probably an enterovirus)	2	epidemic vomiting and diarrhoea, fever
Rotavirus (reovirus family) (gastroenteritis type B)	?	epidemic vomiting and diarrhoea, chiefly of children
Reovirus	3	not clearly established
Adenovirus	> 30	respiratory disease, eye infections
Parvovirus		
Adeno-associated virus	3	associated with respiratory disease of children, but etiology not clearly established

[a] Other stable viruses which might contaminate water are:

SV40-like papovaviruses that appear in the urine. The JC subtype is associated with progressive multifocal leukoencephalopathy.

Creutzfeld–Jakob disease virus. Like scrapie virus, the C–J virus resists heat and formaldehyde. The virus causes a spongiform encephalopathy characterized by severe progressive dementia and ataxia.

were detected in 18% of 200 samples and the average virus concentration was estimated as one infectious unit per 300 litres.[a] Russian investigators have reported the isolation of enteric viruses on several occasions from drinking water in distribution systems;[b] the water treatment plant from which the water came was found to be functioning normally (the process included chlorination) during the periods when viruses were recovered. Viruses have also been isolated from drinking water in South Africa.[c] In a Romanian study, coxsackieviruses were detected in 2 of 65 drinking water samples; the drinking water treatment consisted of flocculation with aluminium sulfate and lime followed by sand filtration.[d] More recently, poliovirus has been detected on several occasions in treated drinking water containing free residual chlorine from a community in the United States.[e] Virological surveys of drinking water supplies are sorely needed to determine whether currently practised water treatment methods are adequate, but unfortunately few laboratories at the present time are equipped or staffed to undertake such studies.

DIFFERENT ROUTES OF TRANSMISSION OF VIRUSES FROM WATER TO MAN

As shown in Fig. 1, there are many potential routes of virus transmission from water back to man. As little as one virus unit infectious for host cell systems in the laboratory is capable, under the proper circumstances, of producing infection in man. Therefore, the presence of even one detectable infectious virus unit in a water supply poses a potential disease hazard. It has been calculated that if a water supply contains as little as one plaque-forming unit of virus per 190 litres of drinking water in a community using 190 million litres per day (0.2% of which is ingested as drinking water), and assuming only a 30% infection rate, 600 individuals would be affected daily by a variety of clinical and sublcinical infections.

In the United States, the number of cases of viral hepatitis caused by waterborne type A virus, which is provisionally classified as an enterovirus, has been increasing in recent years; hepatitis A is now the most prevalent waterborne disease attributable to a specific etiological agent. During this same recent period in the United States, the annual number of cases of typhoid fever, caused by waterborne bacteria, has decreased at least fivefold. This raises questions as to the ability of currently practised water and wastewater treatments to prevent the spread of enteric virus disease.

Documented waterborne outbreaks of virus disease have largely been limited to the agent of infectious hepatitis, mainly because of the explosive nature of these outbreaks and their characteristic symptomatology. Other waterborne virus disease outbreaks are not so easily recognized, and well-documented outbreaks attributable to specific enteric viruses are lacking. There are two main explanations for this:

1. Many of these viruses cause inapparent infections that are difficult to recognize as being waterborne. A person may contract a viral infection by coming in contact with contaminated water, and the virus may actively multiply in the upper respiratory

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[a] COIN, L. ET AL. In: Jenkins, S. H., ed. *Advances in water pollution research.* New York, Pergamon Press, 1966, pp. 1-10.

[b] RABYŠO, E. V. *Gigiena i sanitarija*, **39**: 105-106 (1974).

[c] BERG, G. ET AL., ED. *Viruses in water* (Proceedings of the International Conference on Viruses in Water, Mexico City, 9-12 June 1974). Washington, DC, American Public Health Association, 1975.

[d] NESTOR, I. & COSTIN, L. *Journal of hygiene, epidemiology, microbiology and immunology*, **20**: 137-149 (1976).

[e] HOEHN, R. C. ET AL. *Journal of the Environmental Engineering Division of the American Society of Civil Engineering Division of the American Society of Civil Engineers*, **103**(EE5): 803-824 (1977).

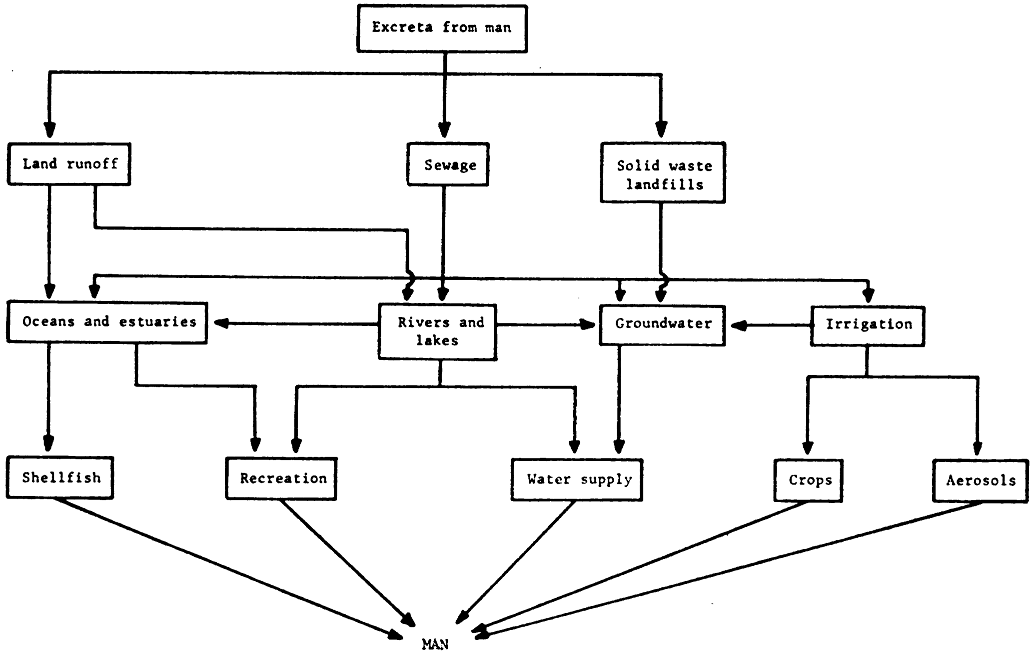

Fig. 1. Potential routes of enteric virus transmission.

tract and intestines without the person developing overt symptoms of the disease. The subject may have only mild respiratory or gastrointestinal distress for a few days or no symptoms at all, yet may act as an effective carrier and transmit the virus by droplet infection or by contaminated fingers to others, who may then develop acute symptoms of the disease.

2. Epidemiological techniques are not sufficiently sensitive to detect low-level transmission of viral diseases through water. Most enteric viruses cause such a broad spectrum of disease syndromes that scattered cases of acute illness would probably have too varied symptoms to be attributed to a single etiological agent.

These reasons may account for the fact that almost 60% of all documented cases of disease attributable to drinking water in the USA are caused by unrecognized or unknown agents. In addition, at present no field-proven method exists for the detection of the agents of infectious hepatitis and nonbacterial gastroenteritis. These difficulties have led to an emphasis on the detection of the readily demonstrable enteroviruses in water as an indication of the possibility of contracting disease from such water.

The occurrence of pharyngoconjunctival fever, caused by an adenovirus, among persons frequenting swimming pools is now widely recognized. Such outbreaks, often among large numbers of swimmers, have usually occurred because of a failure to maintain adequate levels of chlorine in swimming pool water. Little evidence exists at present for the water-borne transmission of other enteric viruses to bathers although, in one recent example, during a coxsackievirus B5 outbreak, the virus was isolated from a swimming area of a lake.

The release of virus into the marine environment through sewage outfalls and polluted rivers has also been an area of concern from the recreational standpoint and as a threat to important shellfish-growing areas. The eating of raw or inadequately cooked shellfish from polluted waters is known to lead to the transmission of hepatitis and gastroenteritis. Hundreds of cases of shellfish-transmitted hepatitis have been reported, and enteric viruses have been detected in oysters taken from both the East and Gulf Coasts of the United States. Poliovirus has been detected in oysters taken from a shellfish-growing area that met accepted bacteriological criteria for shellfish harvesting. Shellfish take in viruses during feeding and accumulate them in their digestive tract tissue, but there is no evidence that the viruses multiply. However, a recent outbreak of viral hepatitis indicated that the causal virus can survive for over 2 months in oysters.

The production of aerosols during spray irrigation with wastewater is another area being investigated at present. The incidence of communicable enteric disease in communal agricultural settlements in Israel practising wastewater spray irrigation with partially treated, nondisinfected oxidation-pond effluent was compared with that in communities that did not practise any form of wastewater irrigation. The incidence of shigellosis, salmonellosis, typhoid fever, and infectious hepatitis was found to be 2–4 times higher in the communities practising wastewater irrigation; no significant differences were found in the incidence of streptococcal infections, tuberculosis, and influenza.[a] Echoviruses were actually isolated from 4 of 12 air samples collected 40 metres downwind of the sprinkler.[b] Clearly, additional work is needed to determine the minimum treatment of wastewater necessary before its use in irrigation.

PERSISTENCE OF VIRUSES IN WATER AND SOIL

Certain enteric viruses can persist for long periods of time in the environment: reported survival times are 2–168 days in tap water, 2–130 days in seawater, 25–125 days in soil, and up to 90 days in oysters.

Previous field and laboratory studies have indicated that large numbers of viruses may be removed from sewage or other waters after percolation through relatively short distances (30–60 cm) in soils of uniform composition. However, more recent research in our laboratory indicates that not all enteric viruses are removed with equal efficiency. The efficiency of virus removal is dependent not only on the type of virus but also on the strain. Polioviruses are removed most readily, while certain strains of coxsackie and echorivuses are only poorly removed.

Removal is believed to be largely due to the adsorption of viruses on to soil particles. However, viruses are not inactivated on the soil particles and may remain viable for many months in the soil matrix, perhaps to be released again when the proper conditions for elution or desorption develop. Work carried out at a spray irrigation site in the United States has indicated that such factors as heavy rainfall may result in changes in factors that affect virus adsorption and desorption, resulting in the release of virus into the groundwater. In this particular instance, viruses were observed to move as a band through the

[a] KATZENELSON, E. ET AL. *Science*, **194**: 944-946 (1976).

[b] TELTSCH, B. & KATZENELSON, E. *Applied and environmental microbiology*, **35**: 290-296 (1978).

soil after heavy rainfall and were detected in wells 3–6 metres below the surface. No viruses had previously been detected in these wells even after several months of sewage application to the land.

There is increasing evidence that viruses are often associated with suspended solids in sewage effluents and natural bodies of water. For example, human enteric viruses have been detected in sludges from activated sludge plants and in marine sediments. It has been demonstrated conclusively that the association of viruses with solids does not result in inactivation; in fact, virus survival appears to be prolonged. Human viruses absorbed to clays and soil particles have been shown to be infectious for both animals and laboratory tissue cultures. Since adsorption is affected by such factors as pH and the presence of salts and soluble organic matter, it has been suggested that viruses could persist in river and marine sediments and not be detected in the overlying water for long periods of time or until they later become desorbed as a result of changes in the above factors. In field studies of coastal waters, 10- to 10 000-fold higher concentrations of enteroviruses have been found in the sediment than in the overlying water.

PROBLEMS ENCOUNTERED IN THE ELIMINATION OF VIRUSES FROM WATER

Processes available for virus removal from water and wastewater have been separated into those involving physical removal and those causing inactivation or destruction of the particle. Processes that involve actual removal include sedimentation, absorption, coagulation and precipitation, and filtration. Conditions that cause inactivation are high pH, chemical oxidation by disinfectants such as halogens, and photo-oxidation by certain dyes in the presence of light. Of these processes, those that bring about virus inactivation are preferable, since with simple removal one is still faced with the disposal of potentially infectious material.

There are several shortcomings in the field evaluation of treatment methods. For example, the number of viruses entering a sewage treatment plant during a 24-hour period varies greatly, making it difficult to coordinate samples temporally for testing the effectiveness of treatments. Primary treatment of wastes, involving only settling and retention before discharge, appears to remove few or no viruses. Any virus removal that occurs during this treatment probably results from the sedimentation of viruses associated with sewage solids. In studies with enteroviruses, removal of up to 90 % has been reported. Recent evidence indicates that rotaviruses, the major cause of gastroenteritis in children, adsorb poorly to sewage solids and would probably be less efficiently removed during sewage treatment.

Under appropriate conditions, viruses are readily absorbed to a wide variety of surfaces, including activated carbon, diatomaceous earth, coal, glass, membrane filters, colloidal organic matter, clays, and soil. Adsorption to such surfaces is reversible by alteration of ionic levels or pH or by the addition of competing organic matter. Activated carbon has been shown to remove viruses, but its capacity is soon reached and virus desorption often occurs as organic substances replace the adsorbed viruses. Sand filters can remove viruses by adsorption onto substances trapped by the sand, but little adsorption occurs onto the sand itself.

The physicochemical treatment of sewage can result in large reductions of virus. Viruses are readily removed by coagulation, and it has been suggested that apart from disinfection this is probably the single most effective chemical procedure for removing viruses from water and wastewater. Alum (aluminium sulfate), lime (calcium hydroxide), and iron salts, as well as polyelectrolytes, have been shown to be capable of removing up to 99.99% of the viruses suspended in water. It has been postulated that coagulation results in the formation of a coagulant–cation–virus complex that settles from solution. The virus is not inactivated by this process and, in fact, such coagulation has been used as a method of concentrating viruses from water. Unfortunately, these results are largely based on studies with enteroviruses. More recent studies with rotaviruses indicate that they adsorb poorly to alum flocs. The high pH that can be attained during lime treatment can also result in very large reductions of virus. If pH levels above 11 are maintained for sufficiently long periods, 99.9% inactivation of the viruses present can result. Large variations in the reported times required for inactivation exist in the literature, and there is a need for a much closer look at factors such as concentration of organic substances, time, and temperature.

Although chlorine treatment has been the mainstay of water disinfection for over 50 years, little is known about the precise mechanism by which it renders viruses non-viable. The effectiveness of chlorine as a viral disinfectant is highly dependent on a number of factors, including temperature, pH, the presence of organic matter, and the physical state of the virus (i.e. adsorbed, aggregated, etc.). Because of the presence of large amounts of organic matter in effluents from activated sludge plants, large reductions of virus are not possible because the chlorine combines with the residual organics. It has been observed that the application of 8 mg of chlorine per litre of sewage effluent resulted in no decrease in virus. With very high doses (40 mg/litre for 10 minutes), 99.9% destruction of virus in sewage has been achieved. However, high doses of chlorine are not only expensive, but also entail the risk of chlorine toxicity to higher forms of life when such effluents are discharged. Also, carcinogenic chlorinated hydrocarbons may be produced. A further complication is the wide variability in the resistance of different enteric viruses to inactivation by chlorine and the enhanced resistance of solid-associated and aggregated viruses. In a study of the resistance of 25 human enteric viruses, the time required for 99.99% inactivation under the same conditions from 3 minutes to 2 hours. A recent report on the development of a progressively more chlorine-resistant poliovirus strain, after a series of repeated sublethal exposures, has created additional concern that chlorine-resistant enteric viruses could arise in nature.

Ozone has been used in many parts of the world as a disinfectant for water and wastewater but little information is available to indicate its virucidal efficiency, especially under field conditions in the presence of organic loads. One of the main arguments against the widespread use of ozone is that it lacks a residual effect, having a half-life of only 25 minutes.

IMPROVEMENT OF METHODS FOR MONITORING VIRUSES IN WATER

There is now a large body of evidence to indicate that enteric viruses are considerably more resistant to various sewage and water treatment methods than either coliform or enteropathogenic bacteria, and therefore the absence of these organisms does not guarantee the absence of a viral disease hazard.

Virus monitoring techniques, although not perfected for all types of water, have been developed to detect virus if only a single infectious unit is present in samples as large as 4000 litres of drinking water. New and improved techniques [a] have also been developed

[a] GERBA C. P. ET AL. *Applied and environmental microbiology.* 35: 540-548 (1978).

for the quantification of viruses in shellfish as well as in sewage and estuary and marine waters.

STANDARDIZATION OF REQUIREMENTS FOR DRINKING WATER

While few laboratories at present have the equipment and experienced personnel to perform these tests, we believe that it is now time to consider the establishment of virus standards for drinking and other waters. It is felt that such guidelines are necessary for the planned use of recycled wastewater for domestic consumption. In June 1974, scientists from around the world met in Mexico City at the International Conference on Viruses in Water, which was sponsored by the American Public Health Association and the World Health Organization, to propose recommendations for the detection and control of waterborne viruses. A prior recommendation that less than one infectious virus unit per 10 gallons be present in recreational water and less than one virus in 100–1000 gallons be present in drinking water was reaffirmed. It was the consensus of this same group that an international body, such as the World Health Organization, should identify provisional methods for determining virus concentrations in water and set up virus standards. While at present there are no official virus standards recommended by the federal government, surveillance and methodology studies on enteric viruses in water supplies of several United States communities are now being conducted. Such studies may form the basis for further recommendation on virus standards.

RÉSUMÉ

Les virus et l'eau

La présence de virus dans l'eau en général ainsi que dans les produits de la mer qui sont consommés constitue un problème de santé publique auquel les milieux médicaux n'attachent peut-être pas encore toute l'attention qu'il mérite. Ceci s'explique sans doute en partie par la diversité des manifestations pathologiques dues aux entérovirus — dont une centaine de types est connue [a] — et à la nature souvent indirecte de la contamination par des personnes ayant été en contact avec l'eau infectée.

Parmi les maladies transmises par l'eau figure maintenant au premier rang, aux Etats-Unis d'Amérique, l'hépatite virale de type A, dont la symptomatologie est caractéristique, alors que l'incidence de la fièvre typhoïde — laquelle est due à une bactérie contaminant l'eau — est au moins cinq fois moins élevée qu'auparavant. On est donc en droit de se demander si les méthodes actuelles de traitement de l'eau et des effluents suffisent à prévenir la propagation des virus pathogènes pour l'homme. Ce problème est d'autant plus grave que le recyclage des eaux usées est appelée à se généraliser dans l'avenir en raison de la demande accrue d'eau à des fins domestiques aussi bien qu'industrielles. Il convient de noter que les entérovirus peuvent persister longtemps dans l'environnement — quelques jours à cinq mois dans l'eau du robinet, l'eau de mer et le sol, deux à trois mois dans les huîtres. Il importe donc d'améliorer les méthodes de détection des entérovirus dans l'eau car, si faible que soit le taux de la contamination virale, celle-ci représente un risque non

[a] Voir tableau 1, page 500.

négligeable de maladie. La densité des virus présents dans les eaux usées dépend naturellement d'un ensemble de facteurs tels que le niveau d'hygiène et le niveau socio-économique, l'incidence de la maladie dans la communauté et l'époque de l'année. Les virus survivent à la chloration et aux autres procédés de traitement secondaire couramment employés aux Etats-Unis, où ils ont été détectés dans les principales rivières. Il est d'ores et déjà démontré qu'ils présentent une résistance aux diverses méthodes de traitement de l'eau et des effluents supérieure à celle des bactéries et on ne peut donc déduire de l'absence de celles-ci qu'il n'existe pas de risque de contamination virale. Des virus ont été isolés dans l'eau de boisson — normalement traitée et distribuée — par des chercheurs de plusieurs pays (Afrique du Sud, France, Roumanie et URSS) et, aux Etats-Unis, des poliovirus ont été détectés à plusieurs reprises dans de l'eau de boisson contenant du chlore résiduel. Il faudrait multiplier les enquêtes de ce type, mais il n'existe encore que peu de laboratoires suffisamment pourvus en personnel et en équipement.

On est déjà bien renseigné sur la présence dans les piscines publiques d'un adénovirus provoquant chez les baigneurs des manifestations pharyngo-conjonctivales fébriles. L'environnement marin est également un grand sujet d'inquiétude aussi bien en raison des risques pour les baigneurs que pour les activités telles que l'ostréiculture (des virus de la poliomyélite et de l'hépatite virale A ont été détectés dans les huîtres provenant de parcs contrôlés sur plusieurs côtes des Etats-Unis). L'irrigation au moyen d'eaux usées — même partiellement traitées — peut provoquer une contamination par aérosols jusqu'à une distance de 40 m du tourniquet; il convient donc de déterminer le traitement minimum des eaux usées compatible avec la sécurité dans ce domaine.

La suppression des virus présents dans les eaux ou effluents par filtration est parfois possible, mais elle n'a pas une égale efficacité pour tous les types de virus — voire pour leurs différentes souches. Les poliovirus ont pu être éliminés de cette manière plus aisément que les Echovirus et le virus Coxsackie. Cette élimination résulte de l'adsorption des virus sur certaines particules du sol, mais ils peuvent être de nouveau libérés dans certaines conditions (en cas de fortes chutes de pluie par exemple). En outre, on a tout lieu de penser que la survie des virus est prolongée lorsqu'ils sont associés à des solides, et des virus humains adsorbés sur des particules de terre glaise ou autres se sont révélés pathogènes pour des animaux et des cultures tissulaires en laboratoire. On suppose que des virus peuvent ainsi persister longtemps dans les sédiments marins ou fluviaux et être libérés lorsque intervient une modification de certains facteurs, notamment le pH.

A la suppression — parfois provisoire — des virus par sédimentation, adsorption, coagulation suivie de précipitation ou filtration serait préférable leur inactivation ou destruction par divers moyens: élévation du pH, oxydation chimique par des désinfectants — notamment halogènes — ou photo-oxydation. L'évaluation des diverses méthodes de traitement est difficile, mais on a récemment établi que les rota-virus, cause principale des gastro-entérites chez les enfants, étaient peu susceptibles d'être retenus au cours du traitement en raison de leur peu d'affinité — pour ce qui est d'une possible adsorption — avec les matières solides contenues dans les effluents, et on n'obtient pas non plus une bonne précipitation avec le sulfate d'aluminium dans le procédé physico-chimique de coagulation. En ce qui concerne l'inactivation, l'augmentation du pH — notamment par le traitement à la chaux — a donné de bons résultats, mais des recherches sur divers facteurs comme la concentration des substances organiques, le temps pendant lequel doit être maintenu un pH élevé et les conditions de température sont encore nécessaires. La chloration de l'eau dont l'efficacité comme moyen général de désinfection de l'eau n'a plus à être prouvée, peut détruire les virus dans certaines conditions. Si les matières organiques résiduelles sont abondantes dans les effluents, la quantité de chlore employée doit être fortement accrue et des problèmes de toxicité peuvent alors se poser. En outre, la résistance des virus au chlore est plus forte s'ils sont associés à des particules solides ou s'ils forment des aggrégats. Cette résistance varie également selon le type de virus, et certaines expériences font redouter en particulier l'apparition d'une souche de poliovirus résistante au chlore. L'emploi d'ozone comme désinfectant n'est pas très répandu en raison de l'absence d'effet rémanent de cet agent.

Les techniques de détection des virus dans les eaux terrestres et marines et dans les fruits de mer ont déjà bien progressé, et il conviendrait maintenant d'envisager l'établissement de normes concernant les virus présents dans l'eau, notamment l'eau de boisson. La Conférence internationale sur les Virus présents dans l'eau, qui s'est tenue à Mexico sous les auspices de l'Association américaine de Santé publique et de l'Organisation mondiale de la Santé, a réaffirmé une recommandation déjà formulée à l'égard de la teneur en virus des eaux de boisson et des eaux récréatives, et elle a convenu qu'une organisation internationale comme l'OMS devrait identifier des méthodes pour déterminer la concentration virale et établir des normes dans ce domaine.